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# BIOLOGICAL BULLETIN

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## ON THE RHYTHMICAL SUSCEPTIBILITY OF DEVELOPING SEA URCHIN EGGS TO HYPERTONIC SEA WATER.

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In a recent communication M. Herlant<sup>1</sup> attempting an analysis of Loeb's method of artificial parthenogenesis concludes that (1) the fatty acid treatment gives rise to the rhythmical activity of the centrosome but never to normal divisions of the egg; (2) the treatment of the eggs with hypertonic sea water causes the formation of accessory asters and is necessary to complete the causes for normal division; (3) the optimum results are obtained by applying the hypertonic solutions at certain intervals after fatty acid treatment, viz., 30 and 70 minutes and possibly 115-120 minutes, while with 40-50 and 95-100 minute intervals marked minima are shown.

As to the first generalization, Herlant ignores the fact that in *Strongylocentrotus* and *Arbacia* the fatty acid treatment alone may cause normal segmentation. If the eggs of *S. purpuratus* are kept at a low temperature (5°-10°) after acid treatment alone, they divide regularly and may reach the morula stage.<sup>2</sup> In *Arbacia* eggs, normal segmentation may take place after acid treatment without subsequent treatment with the hypertonic solution, but does not as a rule proceed beyond the two-cell stage. On the other hand hypertonic treatment alone may cause *Arbacia* eggs to segment and develop into swimming larvæ, while it brings about only early segmentation stages in the eggs of *Strongylocentrotus*.

<sup>1</sup> M. Herlant, *Comptes Rendus de l'Academie*, T. 158, p. 1531.

<sup>2</sup> J. Loeb, "Artificial Parthenogenesis and Fertilization," p. 76.

Herlant apparently assumes that the action of the hypertonic solution must be subsequent to the acid treatment, that to be effective such action must occur in a certain phase of the rhythmical activity of the centrosome. Since in *Strongylocentrotus purpuratus* treatment of the eggs with the hypertonic solution may precede that with acid sea water by as much as a forty-eight hour interval with the result that normal parthenogenetic larvæ are formed,<sup>1</sup> Herlant's contention is not justified. Furthermore, treatment of the eggs with small quantities of KCN or depriving them of oxygen, may replace hypertonic treatment after fatty acid. Now lack of oxygen or the repression of oxidations does not cause aster formation, but on the contrary suppresses it. In fact it has even been shown that in *Arbacia* the first steps in development induced by the acid treatment may be reversed and the egg returned to its resting stage with its original possibilities of fertilization, simply by withholding oxygen from such an egg or by treating it with KCN.<sup>2</sup> In view of these facts, it seems evident that Herlant's conclusion that the hypertonic solution is a necessary factor in artificial parthenogenesis because it controls aster formation, does not hold.

As to the rhythmicity in effectiveness of the hypertonic treatment which Herlant found in his experiments, it seemed possible that the relation between the time spent in normal sea water after acid treatment and the time in the hypertonic solution might have a bearing upon the question. Six years ago the present writer found, in working with the eggs of *Strongylocentrotus purpuratus*, that if the exposure to normal sea water in such an experiment be lengthened, the subsequent treatment by hypertonic sea water must be shortened to secure optimum results.<sup>3</sup> Repetitions of the experiment at Woods Hole during the past summer, however, have indicated that the relation does not exist for *Arbacia*.

Table I. shows the results of dividing a lot of *Arbacia* eggs, after fatty acid treatment, into three parts which remained in normal sea water, 5, 25 and 90 minutes respectively, before being put into hypertonic sea water. In each case a portion

<sup>1</sup> J. Loeb, *Journ. Exp. Zool.*, vol. 15, p. 201.

<sup>2</sup> J. Loeb, *Science*, N. S., Vol. 38, p. 749.

<sup>3</sup> J. Loeb, "Artificial Parthenogenesis and Fertilization," p. 96.

of each lot was removed from the latter solution after 17½, 20, 25, 30, 35, and 40 minutes, and allowed to develop in normal sea water. The percentages given in the table show the degree of blastula development in each culture. Repetitions of the experiment showed no significant variation in the optimum exposure to the hypertonic solution with changes in the time the eggs remained in the normal sea water after acid treatment.

TABLE I.

$T^{\circ} = 20^{\circ} - 22^{\circ}$ .

Eggs Remained in Normal Sea Water, After Butyric Acid Treatment	Percentages of Eggs Develop into Blastulæ After Exposure to Hypertonic Sea Water for					
	17½ Min.	20 Min.	25 Min.	30 Min.	35 Min.	40 Min.
5 minutes.....	—	1%	5%	13%	24%	18%
25 " .....	—	8%	18%	24%	20%	8%
90 " .....	—	—	—	1%	3%	1%

In order to determine if the eggs of *Arbacia punctulata* which had been treated with acidulated sea water, showed a rhythmicity in sensitiveness to the hypertonic solution, such as Herlant's experiments with the eggs of *Paracentrotus lividus* indicated, the following experiments were carried out. The eggs of several sea urchins were collected, treated with sea water made acid by the addition of 2 c.cm. N/10 butyric acid to 50 c.cm. of sea water. After remaining in this solution for from 2 to 2½ minutes the eggs were transferred to normal sea water. At the end of 5-minute intervals lots were removed to finger bowls containing hypertonic sea water [50 c.cm. sea water + 8 c.cm. 2½ M (NaCl + CaCl<sub>2</sub> + KCl)]. After remaining in the hypertonic sea water for 25 minutes the eggs were put into normal sea water and allowed to develop. The percentages of advanced morulae or non-swimming blastulæ were determined by counting random fields. The following table (Table II.) gives a typical result, showing optimum effects when the eggs were put into the hypertonic solution 40, 60, 90–100 and 115–125 minutes after acid treatment. The rhythmical character of the result is obvious from Curve I., where the ordinates indicate the percentage of larvæ formed, while the abscissæ indicate the time which elapsed between acid treatment of the eggs and their exposure to hypertonic sea water.

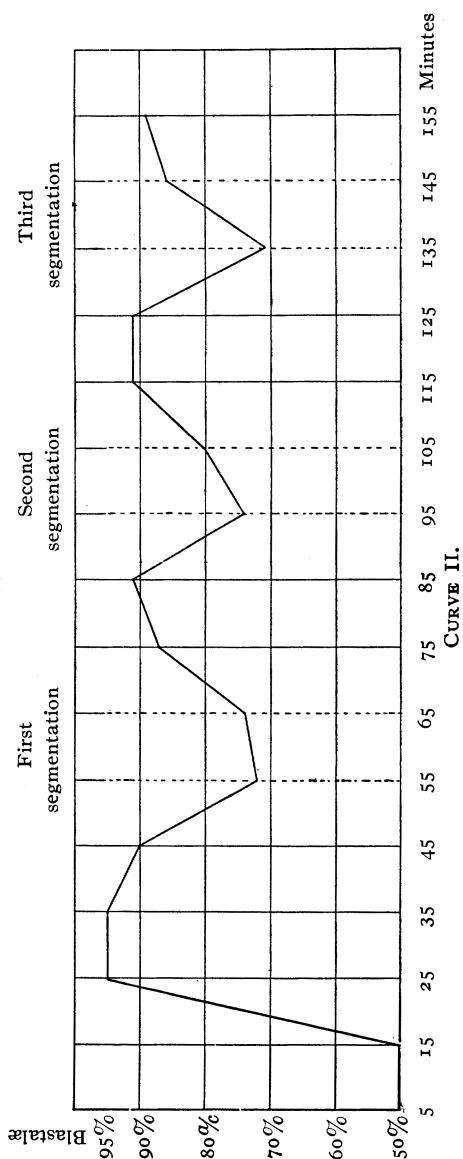
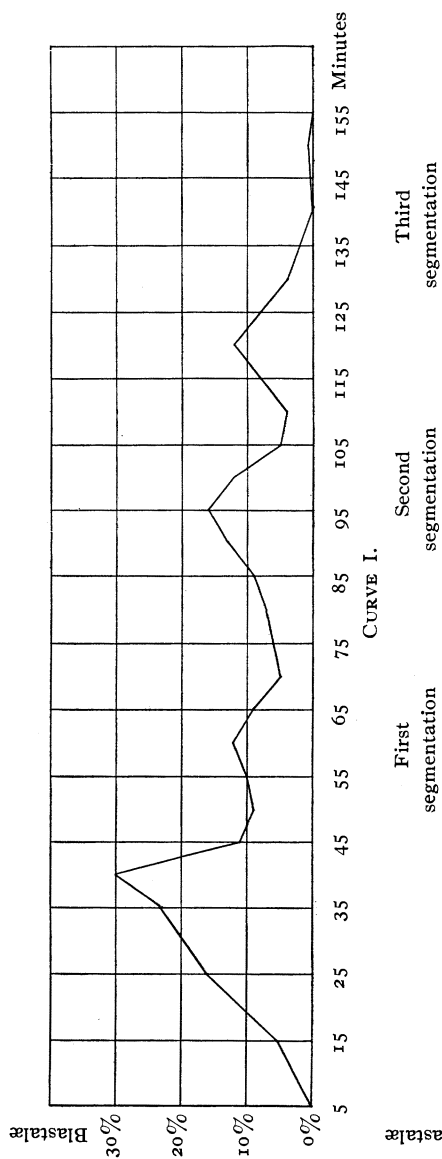


TABLE II.

 $T^{\circ} = 19^{\circ} - 21^{\circ}$ . Hypertonic exposure = 25 minutes.

Time in normal sea																			
water after acid																			
treatment.....	5	15	25	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110
Blastulæ per cent. ...	-	5	16	23	30	11	9	10	12	9	5	6	7	9	13	16	12	5	4
Time in normal sea																			
water after acid																			
treatment.....	115	120	125	130	140	150	165	175	185										
Blastulæ per cent. ...	8	12	8	4	0	<1	0	1	0										

It seemed possible that the normally fertilized eggs also might show a rhythmical susceptibility to hypertonic sea water. Especially did this seem probable in view of Lyon's experiments. He found that normally fertilized eggs of *Arbacia* gave alternating maxima and minima of susceptibility to heat, cold and lack of oxygen; and that  $\text{CO}_2$  production was greatest at the time of cytoplasmic division.<sup>1</sup> E. G. Spaulding has shown a rhythmical susceptibility and immunity of fertilized *Arbacia* eggs to the effects of ether, HCl, KCl and NaCl. He found a rise in immunity up to the time segmentation begins, followed by a sharp decrease during cleavage, with a marked rise at the end of cleavage.<sup>2</sup> A. P. Mathews found an approximate rhythmicity in the behavior of *Asterias* eggs toward KCN solutions.<sup>3</sup>

In my own experiments the *Arbacia* eggs were fertilized in one finger bowl and at the end of each ten-minute interval a lot was removed to the hypertonic solution and kept there for 40 minutes, after which the eggs were returned to normal sea water to develop. The results of a number of such experiments indicate (Table III., Curve II.) that the maximal susceptibility occurs just after fertilization (5-15 minutes) and immediately before and during each cytoplasmic division, and that the maximal resistance is shown 35-45 minutes after fertilization and just after each division. This corresponds to Lyon's statement regarding the effects of heat upon dividing eggs, viz., that *Arbacia* eggs are especially sensitive to heat just before division and that they are most resistant after division.

<sup>1</sup> E. P. Lyon, *Am. Journ. Physiol.*, Vol. 7, p. 56, and Vol. 11, p. 52.<sup>2</sup> E. G. Spaulding, *BIOL. BULL.*, Vol. 6, p. 224.<sup>3</sup> A. P. Mathews, *BIOL. BULL.*, Vol. 11, p. 137.

TABLE III.

 $T^{\circ} = 20^{\circ}$ .

Time in normal sea water

after fertilization.....	5	15	25	35	45	55	65	75	85	95	105	115	125	135	145	155
Blastulæ per cent.....	50	50	95	95	90	72	74	87	91	73	80	91	91	71	86	89

The character of the curve constructed from Table III. differs from that made from the data of Table II. It will be noted that in the latter case there is an early maximum and minimum, and a slight secondary maximum reached at about the time cleavage would take place if the eggs had been normally fertilized. The dissimilarity may be due to the fact that the hypertonic solution may have two effects on developing eggs, viz., beneficial and injurious. We have seen that the acid treatment alone leads to the early stages of development, and that if the temperature is kept low the eggs of *S. purpuratus* develop to the early blastula stage. But before the gastrula stage is reached, the embryos go to pieces. To all appearances the early morula stages are quite normal. Loeb has suggested that cleavage may be accompanied by the production of toxic substances which, accumulating with each successive division, cause the larvæ to sicken and die. The injurious effects of these substances may be prevented by treatment of the egg with a hypertonic solution, or the formation of the injurious substances may be inhibited by long hypertonic treatment before membrane formation. It has been shown that simply by preventing oxidations for a time instead of treating the eggs with hypertonic sea water, normal development may be secured. Hence, the hypertonic solution or lack of oxygen exercises a beneficial or curative effect on parthenogenetically developing eggs and allows the embryo to develop to maturity.

On the other hand there is an optimum time for the continuance of the action of the hypertonic solution, and if the exposure is continued longer the solution acts deleteriously and as a result development stops and the egg disintegrates. It becomes clear then that the hypertonic treatment may have one of the two opposite effects upon the egg, *i. e.*, beneficial or injurious, depending upon the duration of the treatment, or in other words upon the condition of the egg when treated.

In normal fertilization the sperm cell which fertilizes the egg accomplishes two things of immediate importance, viz., starts division and prevents the production of toxic substances during cleavage or inhibits their action. It is therefore impossible for the hypertonic sea water to exercise its protective action upon the normally fertilized egg. It can affect the egg only injuriously. Our experiments show that this injurious action of the hypertonic solution is most pronounced just preceding and during cytoplasmic division, and that such action is very slight immediately afterward.

In the case of artificial parthenogenesis the hypertonic treatment is much shorter than in the experiments just described, and hence we may consider its injurious effects excluded. The Curve I. representing the results of Table II. shows the rhythmicity of the beneficial effect of the hypertonic treatment, while the curve constructed from Table III. indicates only injurious effects. There is therefore no reason why the two curves should be identical in character, although each shows a rhythmicity of susceptibility to the action of hypertonic sea water.

According to Loeb the artificial membrane formation in artificial parthenogenesis starts the chemical phenomena which give rise to the process of cell division and development; but the process is incomplete or abnormal and leads to the disintegration of the egg unless a second treatment is added, usually a treatment with hypertonic sea water. Since, by the membrane formation, chemical or physico-chemical changes induced in the egg are rhythmical, it is intelligible that it should make a difference in which stage of the cycle the treatment with the hypertonic solution is supplied. This is presumably the explanation of Herlant's observation.

In conclusion I wish to express my best thanks to Dr. Frank R. Lillie for so generously giving to me the privileges of the Marine Biological Laboratory at Woods Hole, and to Dr. Jacques Loeb for much helpful advice and criticism.